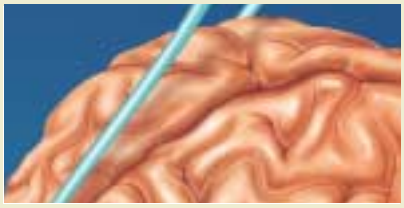
abstract



To date, four genes responsible for Alzheimer disease (AD) have been identified. However, in about 50% of the familial AD cases, there is no known cause of the disease. The majority of AD cases are sporadic with onset after 65 years of age. The apolipoprotein E gene is the only well-replicated risk factor for late-onset AD. Up to 5% of AD cases are early-onset AD, for which genetic analyses have found three causal genes: β-amyloid precursor protein, presenilin-1 and presenilin-2. Treatment and diagnostic strategies based on genetic knowledge are now about to reach the clinic.

Key words: *Alzheimer disease, presenilin, gene, βAPP, apolipoprotein E.*

Genetics of Alzheimer Disease: Progress and Application

Ekaterina Rogaeva, PhD, *Assistant Professor, Department of Medicine, University of Toronto, Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON.*

Introduction

Alzheimer disease (AD) is a progressive dementia and is the fourth leading cause of death in industrialized countries. AD brain pathology is characterized by neuronal loss, intra-neuronal tau-accumulation and extracellular amyloid plaques. The plaques consist mainly of $A\beta_{40/42}$ peptides generated by cleavage of the β-amyloid precursor protein (βAPP) (Figure 1). The longer and more neurotoxic isoforms, $A\beta_{42}$, appear to be elevated in the brains of individuals affected with either sporadic or familial AD, implying that they have a shared pathogenetic mechanism. Most likely, the abnormalities in βAPP processing trigger the formation of neurofibrillary tangles from hyper-phosphorylated tau, although tau-accumulation itself has neurotoxic consequences. Tau is genetically involved in frontotemporal dementia, but not in AD.

AD is a complex disorder with multiple interacting causative and modifying genetic and environmental factors (Figure 2). Twin studies have found the concordance rate for AD among monozygotic twins to be 78% versus 39% among dizygotic twin pairs, indicating a strong genetic influence.[1] The majority of late-onset AD cases (after 65 years of age) are sporadic; the apolipoprotein E (ApoE) is the only well-replicated risk factor for this form of AD.[2] Up to 5% of cases are associated with early-onset AD. The disease in these families is often transmitted as a pure genetic, autosomal dominant trait.[3] To date, genetic analyses of such pedigrees have found three causal genes: βAPP;[4] presenilin 1 (PS1);[5] and presenilin 2 (PS2) (Figure 3).[6]

The aims of this review are to give an update on the pathological consequences of mutations in AD genes, to indicate how this knowledge could be applied in clinical practice, and to summarize the strategies on the search for new AD genes.

The βAPP Gene

Thirteen AD-associated mutations have been found in the βAPP gene located on chromosome 21 (age at onset 40–65 years). Another seven substitutions either have a questionable pathogenic nature or are associated with a different stroke-related, but amyloid-dependant, pathology.[7] The differences in clinical presentation of the disease could be explained by the mechanisms responsible for the proteolytic cleavage of βAPP protein (Figure 1).

βAPP can be processed by at least two separate pathways. One involves α-secretase cleavage within the Aβ peptide sequence. The other pathway requires proteolysis by β- and γ- secretases to generate $A\beta_{40\text{-}43}$ peptides. The mutations are clustered near the α-, β- or γ-secretase cleavage sites, demonstrating that they have a direct effect on βAPP processing.[8] The majority of the mutations either lead to an elevation of the $A\beta_{42}$ peptides, or to an increase of both short and long forms of Aβ. In contrast, the Ala692Gly mutation reduces α-secretase cleavage but increases the variety of the Aβ species.[9] Furthermore, Val715Met and Glu693Gly reduce total Aβ production,[10,11] indicating that the overall ratio of $A\beta_{42}$ to the other Aβ species may be a more relevant indicator of AD pathology than the absolute level of $A\beta_{42}$ or total Aβ.

PS1 and PS2 Genes

Mutations in the PS1 gene, located on chromosome 14, are responsible for the most aggressive form of AD (age at onset 16–65 years) and account for up to 50% of all early-onset AD cases.[2] The majority of the 137 known PS1 mutations are missense substitutions; only one innocent coding variation has been reported to date (E318G).[7] One of the most frequent PS1 mutations is E206A, which was observed in 18 unrelated Caribbean Hispanic people.[12]

In contrast, only nine AD families with variable age at onset (range between 40 and 85 years) are associated with the PS2 gene located on chromosome 1.[13] Presenilins share amino acid and structural similarities and perform similar activities as the components of independent large complexes involved in the α-secretase cleavage. The concept that changes in βAPP processing are central to AD pathology won further support after the discovery that mutations in the presenilins cause the overproduction of the $A\beta_{42}$.[14]

The presenilins have a very complex functional profile as integrators of several signaling pathways. In addition to βAPP processing, presenilins are essential for the proteolytic cleavage of Notch1[15] and several other transmembrane proteins, which raise concerns for therapeutic intervention based on inhibitors of α-secretase activity. It is possible that a dysfunction of these pathways can contribute to neurodegeneration.[3] For instance, the L166P, which causes onset of AD in adolescence, not only induces high increase of $A\beta_{42}$ production but also substantially impairs Notch1 signaling. Intriguingly, the insertion of R352 that decreases cleavage of both βAPP and Notch1 was described in a family with a phenotype resembling frontotemporal dementia.[16] However, without confirmation by autopsy, this diagnosis remains questionable.

Another type of phenotypic heterogeneity was observed in 15 PS1 families associated with spastic paraplegia (SP), characterized by progressive weakness of the lower limbs. Interestingly, the brain pathology of these cases differs from the typical picture for AD. Mature plaques are scarce; instead, there are diffuse, Aβ-positive cotton wool plaques without a congophilic core and with only minor neuritic pathology and markers of inflammation.[17]

A PS1 mutation by itself is unlikely to be responsible for the variant phenotype.[3] The age at onset in these cases ranges from 24–51 years, indicating that the severity of AD does not explain unusual phenotype. The mutations are not clustered in a particular PS1 region, and an identical PS1 mutation has been found in a family with the variant AD phenotype, as well as in

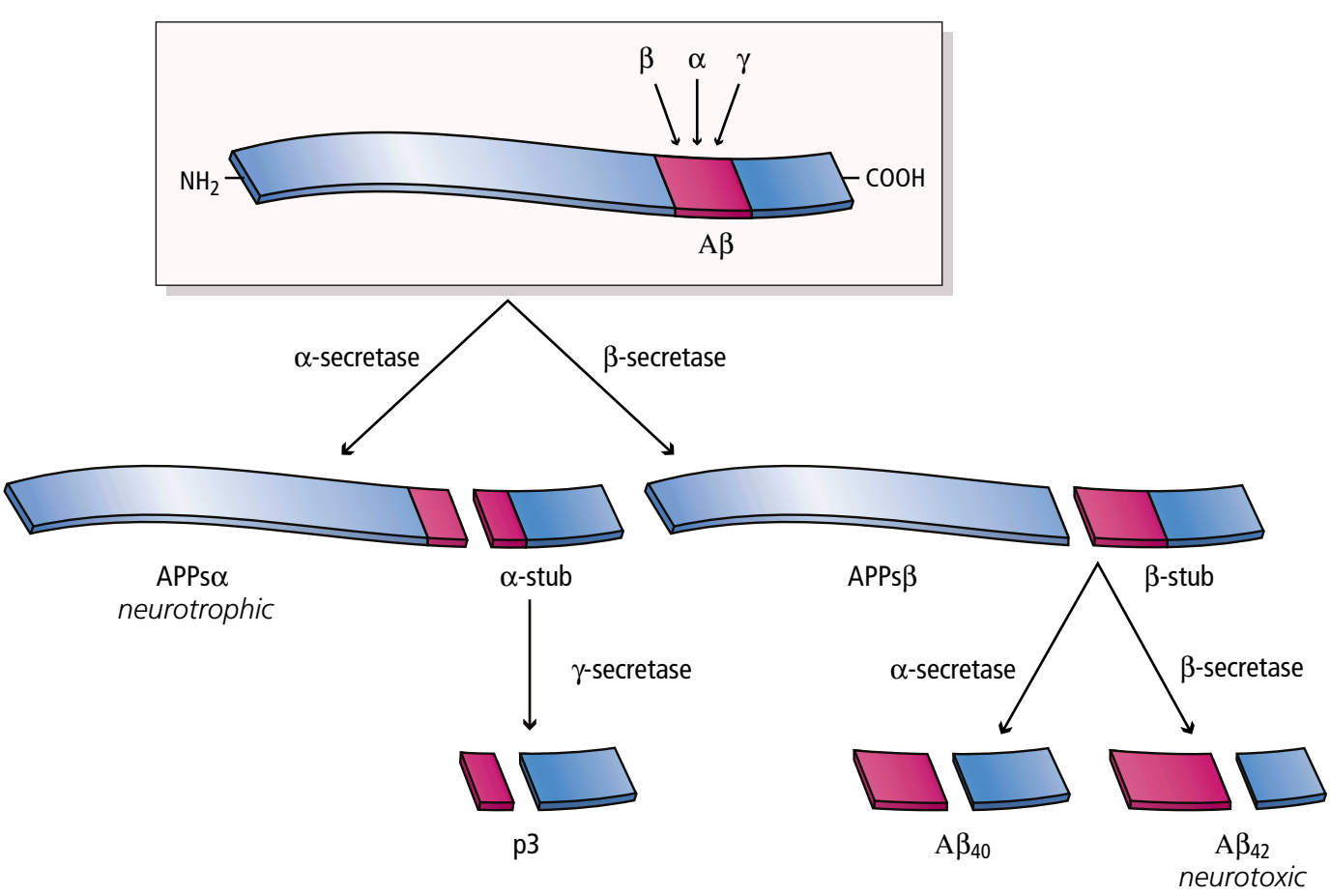


Figure 1. The βAPP protein is processed by two proteolytic pathways, one of which generates Aβ.

a family that presented with typical AD.[17,18] These observations argue in favour of the existence of a modifier in variant AD families. We have excluded that this modifier effect arises from mutations in known SP genes or from a second mutation in the other AD genes.[19] The search for the factors responsible for the variant AD phenotype may shed light on the biochemical pathways influencing amyloid deposition.

Ongoing Search for Alzheimer Disease Risk Factors

In at least half of the AD cases, there is no known cause of the disease. The first step toward finding a new AD gene is identification of linkage to a certain chromosome.[20-22] Pedigrees with AD are the engine for the discovery of new genes: the families with two or more living, affected individuals can be analysed jointly. Linkage analysis relies on the principle that DNA sequences that are close together tend to be inherited together. If the inheritance of innocent DNA variations (markers) is followed through families, a researcher can find a sequence that co-inherits more often with the disease than by chance. Such a result means that the marker's sequence is in the same chromosomal region as the mutation causing the disease.

Another way to identify a genetic risk factor is to select gene by function and then to test its sequence variation(s) for association with the disease. For instance, the secretases involved in the cleavage of Aβ are plausible biochemical candidate genes for AD. The β-secretase (BACE) was cloned recently; however, our study established that it is not genetically involved in AD.[23] Many candidate genes have been reported to be associated with AD. The regulation of the transcription of known AD genes might be an important factor in the disease.[24] However, the only well-replicated risk factor for late-onset AD is the APOE gene on chromosome 19,[2] which expresses a lipoprotein involved in cholesterol metabolism and perhaps in the clearance of Aβ.

The three APOE isoforms are encoded by alleles ε2, ε3 and ε4. The ε4 variation is significantly over-represented in AD subjects, to 40% from 15% in the general population.[2] The mean age of onset of AD is less than 70 years among the ε4/ε4 population, but over 90 years for the ε2/ε3 population.[25] The APOE-ε4 may not be sufficient to cause AD, but rather may provoke neuronal degeneration by preventing normal repair mechanisms. Notably, up to 68% of AD cases do not have an APOE-ε4 allele, indicating that additional factors are involved in the late-onset form of the disease.

Genetic Testing of Alzheimer Disease in Clinical Practice

Without precise biological indicators that precede or accompany the cognitive decline, diagnostic confirmation of AD requires postmortem examination. Genetic testing, in addition to the diagnostic value, may prove significant in identifying at-risk relatives who could be candidates for prophylactic therapies for AD. Until recently, in the absence of effective AD treatments, the lack of pre-symptomatic diagnostics was not critical. However, new therapies for AD are currently in pre-clinical development.

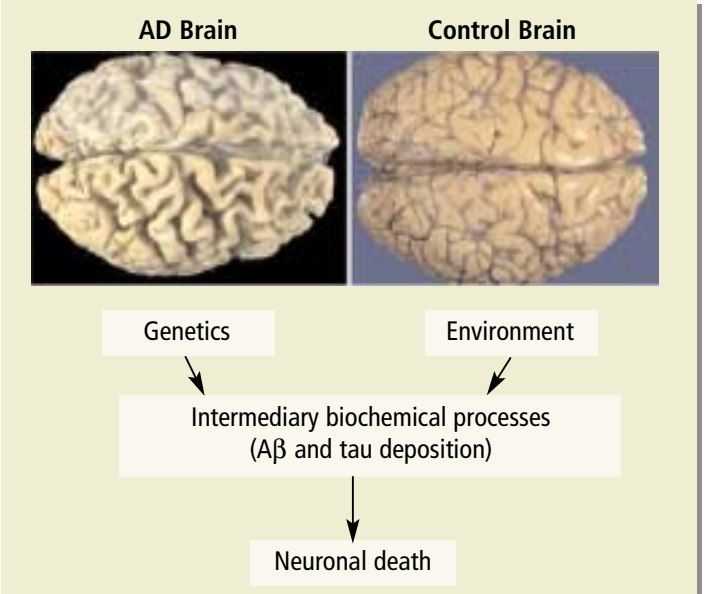
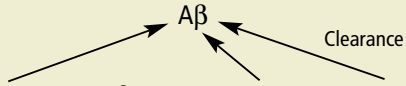


Figure 2. Pathobiology of Alzheimer disease: Aβ accumulation initiates a biochemical cascade leading to neuronal death and clinical symptoms of AD. The progressive neuronal dysfunction and death that characterize AD can involve multiple mechanisms that are modulated by a number of genetic or environmental factors and age-related processes.

PS1 mutations inevitably cause disease and are relatively frequent, making this gene suitable for genetic testing. We estimated the frequency with which PS1 mutations were actually found when clinicians had a high index of suspicion of familial AD: 11% of 414 referral cases can be explained by a PS1 mutation. Patients with a positive PS1 test were significantly younger (46±11years) than patients with negative PS1 tests (60±11years).[26] Therefore, such screening is likely to be especially productive when directed toward persons with a positive family history and age at onset before 60 years. However, there are some concerns for genetic counselling, such as the importance of confirming the pathological nature of novel PS1 mutations. In the absence of evidence of co-segregation of a novel mutation with AD in small families, the analysis of $A\beta_{40-42}$ levels in cultured cells could provide biochemical support for the pathological significance of a mutation.

The APOE gene itself is not useful for pre-symptomatic testing since not all ε4 carriers will develop the disease and ε4-association is not entirely specific to AD. Nevertheless, in the future APOE can be used in combination with other yet to be discovered AD risk factors. Indeed, the late-onset form of AD is a complex genetic trait with the potential involvement of many genes that could have weak effects on their own, but may lead to AD as a result of a combined effect. In parallel with genetic studies, there is a demand to develop appropriate statistical and ethical paradigms for using this type of information.

Amyloid Plaques



	PS1	βAPP	PS2	APOE e4 allele	New Loci
	chr 14	chr 21	chr 1	chr 19	chr 10
Age of Onset	16–65	45–65	40–85	>65	chr 12
Frequency	18–50%	<5%	<5%	~50%	chr 9
	Early-onset AD			Late-onset AD	

Figure 3. Genetic determinants of Alzheimer disease: defects in four genes affect different steps of a complex metabolic pathway of which APP is the earliest known target. Aberrant production, aggregation and/or the poor clearance of toxic Aβ peptides are central to AD neuropathology.

Conclusion

Genetic studies have proven to be an effective means of developing our understanding of AD. This knowledge is opening the way for the development of safe and effective therapies based on blocking Aβ production, inhibiting its aggregation or accelerating its removal. The genetic data may assist in the identification of individuals predisposed to AD while the neuronal damage is still negligible. ◆

No competing financial interests declared.

References

1. Bergem AL, Engedal K, Kringlen E, The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. Arch Gen Psychiatry 1997;54:264-70.
2. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
3. Rogaeva E. The solved and unsolved mysteries of the genetics of early-onset Alzheimer's disease. J Neuromol Med 2002;2:1-10.
4. Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991:349:704-6.
5. Sherrington R, Rogaev EI, Liang Y, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995:375:754-60.
6. Rogaev EI, Sherrington R, Rogaeva EA, et al. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 1995;376:775-8.
7. Alzheimer Disease Mutations Database. http://molgen-www.uia.ac.be/ADMutations/.
8. Hardy J. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci 1997;20:154-9.
9. Haass C, Hung AY, Selkoe DJ, et al. Mutations associated with a locus for familial Alzheimer's disease result in alternative processing of amyloid beta-protein precursor. J Biol Chem 1994; 269:17741-8.
10. Ancolio K, Dumanchin C, Barelli H, et al. Unusual phenotypic alteration of beta amyloid precursor protein (betaAPP) maturation by a new Val-715 --> Met betaAPP-770 mutation responsible for probable early-onset Alzheimer's disease. Proc Natl Acad Sci USA 1999;96:4119-24.
11. Nilsberth C, Westlind-Danielsson A, Eckman CB, et al. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat Neurosci 2001;4: 887-93.
12. Athan ES, Williamson J, Ciappa A, et al. A founder mutation in presenilin 1 causing early-onset Alzheimer disease in unrelated Caribbean Hispanic families. JAMA 2001;286:2257-63.
13. Sherrington R, Froelich S, Sorbi S, et al. Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. Hum Mol Genet 1996;5:985-8.
14. Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 1996;2:864-70.
15. De Strooper B, Annaert W, Cupers P, et al. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. Nature 1999;398:518-22.
16. Amtul Z, Lewis PA, Piper S, et al. A Presenilin 1 mutation associated with familial frontotemporal dementia inhibits gamma-secretase cleavage of APP and notch. Neurobiol Dis 2002;9:269-73.
17. Crook R, Verkkoniemi A, Perez-Tur J, et al. A variant of Alzheimer's disease spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. Nat Med 1998;4:452-5.
18. Hiltunen M, Helisalmi S, Mannermaa A, et al. Identification of a novel 4.6-kb genomic deletion in presenilin-1 gene which results in exclusion of exon 9 in a Finnish early onset Alzheimer's disease family: an Alu core sequence-stimulated recombination? Eur J Hum Genet 2000;8:259-66.
19. Rogaeva E, Bergeron C, Sato C, et al. PS1 Alzheimer's Disease family with spastic paraplegia: the search for a gene-modifier. Neurology. In press.
20. Pericak-Vance MA, Bass MP, Yamaoka LH, et al. Complete genomic screen in late-onset familial Alzheimer disease. Evidence for a new locus on chromosome 12. JAMA 1997;278:1237-41.
21. Kehoe P, Wavrant-De Vrieze F, Crook R, et al. A full genome scan for late onset Alzheimer's disease. Hum Mol Genet 1999;8:237-45.
22. Rogaeva E, Premkumar S, Song Y, et al. Evidence for an Alzheimer disease susceptibility locus on chromosome 12 and for further locus heterogeneity. JAMA 1998;280:614-8.
23. Nicolaou M, Song YQ, Sato CA, et al. Mutations in the open reading frame of the beta-site APP cleaving enzyme (BACE) locus are not a common cause of Alzheimer's disease. Neurogenetics 2001;3:203-6.
24. Theuns J, Van Broeckhoven C. Transcriptional regulation of Alzheimer's disease genes: implications for susceptibility. Hum Mol Genet 2000;9:2383-94.
25. Roses AD. Apolipoprotein E and Alzheimer's disease: The tip of the susceptibility iceberg. Ann N Y Acad Sci 1998;855:738-43.
26. Rogaeva EA, Fafel KC, Song YQ, et al. Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations. Neurology 2001;57: 621-5.